Genetic Variability among Sugarcane Genotypes Based on TRAP Markers of Candidate Drought Genes and Some Agronomic Traits

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Abstract:

The study was focused on evaluation the performance of 12 sugarcane (*Saccharum officinarum* L.) genotypes under drought stress. In addition, the genetic variability among the tested genotypes were studied using Target Region Amplification Polymorphism (TRAP). markers based on polymorphism in Aquaporin (Aqua) and Dehydration Binding Factor (DBF) candidate drought genes. Stalk length, diameter, number, volume and Brix% were sharply affected by drought stress in comparison to the control treatment, however, highly significant differences between the tested genotypes were observed. Drought tolerance index (D.T.I) indicated that EI 8-129 followed by EI 24-3 were the most tolerant genotypes, while NCO310 and G2000 -79 were the most drought sensitive. Drought stress decreased chlorophyll contents in most genotypes except in EI 264-2, G84 – 47 and EI 266-2 the chlorophyll was increased.

The Aqua gene showed high number of amplified and polymorphic DNA fragments than DBF locus with 71.25% overall polymorphism. The largest number of amplified fragments was generated by Aqua + arbit.2 primer combination, and the lowest amplified with the DBF + arbit.1. The significant correlation between D.T.I. and number of amplified fragments from Aqua and DBF indicated that these loci play an important role in sugarcane drought tolerance. The lowest genetic similarity observed between the drought tolerant and the most sensitive genotypes illustrating that crosses between these genotypes would probably result in the highest variability for drought among the genotypes sampled. Cluster analysis based on TRAP markers of Aqua and DBF loci displayed that some genotypes that have common parents were grouped together and those displayed drought tolerance were also grouped together. The results reflected the feasibility and effectiveness of Aqua and DBF loci as molecular markers for drought tolerance in sugarcane.

Keywords*:* *Sugarcane, Saccharum officinarum, genetic variability, TRAP markers, Aquaporin, Dehydration binding factor DBF, drought gene family, UPGMA cluster analysis.*

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Introduction:

Sugarcane is probably the most genetically complex crop for which genome mapping has been attempted. Sugarcane cultivars are polyploid, aneuploid, and interspecific hybrids between the domesticated species *Saccharum officinarum* and the wild relative *S. spontaneum*. Cultivars chromosome numbers range from 100 to 130 with ca. 10% contributed by *S. spontaneum* (Sreenivasan *et al*., 1987). The selection of desirable combinations of characters at these complex-levels of ploidy is much more laborious and required larger populations that at the diploid level.

Water is the most significant limiting factor to the productivity and crop yield of sugarcane *Saccharum* spp. L. (Singh and Reddy, 1980). Increasing world population and water pollution along with fixed water supplies are likely to aggravate the effects of drought on global sugar food security. Drought has several impacts on the success of the early life stages of plants. In sugarcane, the formative growth stage of sugarcane (60th to 150th day) is the most water-demanding phase; plants are the most severely affected by water stress (Singh and Reddy, 1980; Venkataramana *et al.*, 1983&1986; Nadiu and Venkataramana, 1987). Juice quality of sugarcane was shown to be affected by soil water content during the early stage of growth. Generally, there is a variety of morphological, physiological, and biochemical responses and adaptations to the effects of drought stress (Mousa *et al*. 2012).
Breeding for drought resistance is complicated as drought is a complex trait involving a battery of genes and regulatory elements and sugarcane being a complex polyploid. Though response of elite genotypes of sugarcane has been studied (Hemaprabha *et al*, 2004), a lot more on the underlying genetic factors involved in plant responses to drought stress need to be understood in order to provide a solid foundation to breed plants with improved drought tolerance (Sanchez *et al*, 2002). One of the most productive approaches to establishing the basic responses of plants to drought stress involve studying candidate gene. The candidate genes, or DNA sequences with predicted function, are used as molecular markers to associate with phenotypes expressed in segregating populations or genetic stocks (Huh *et al* .2001; Thorup *et al.* 2000). The identification of molecular markers based on such novel genes will provide us the basis of effective engineering strategies to improve stress tolerance (Cushman & Bohnert, 2000). Among the most recent is the Target Region Amplification Polymorphism (TRAP) introduced by Hu and Vick (2003). TRAP is a novel polymerase chain reaction (PCR)-based marker system that takes advantage of the available Expressed Sequence Tag (EST) database sequence information to generate polymorphic markers targeting candidate genes. Since TRAP is based on PCR technology using anchored and arbitrary primers to amplify coding regions in the genome, the resulting polymorphism should be reflective of diversity within functional genes.

The present study was focused on evaluation of 12 sugarcane genotypes under drought stress. TRAP analysis were used to assess the genetic variability among sugarcane genotypes based on Aquaporin (Aqua) and dehydration binding factor (DBF) candidate genes involved in drought tolerance.

Materials and Methods:

The present investigation was carried out at the Experimental farm of Genetics Department, Faculty of Agriculture, Assiut University, Assiut, Egypt, to evaluate the performance of 12 sugarcane (*Saccharum officinarum* L.) genotypes under drought stress. In addition, the ge-

netic variability among the tested genotypes were studied using TRAP markers based on polymorphism in Aquaporin (Aqua) and Dehydration Binding Factor (DBF) candidate drought genes. The tested varieties were kindly obtained from the research station of Sugar and Integrated Industries Company which namely; EH 16-9, EI 8-129, EI 264-2, EI 266-2, EI 24-3OR2, G84 – 47, G99-103, G2000 -79, G.T.54C-9, NCO310, N26 and PH8013 (Table 1).

Drought stress:

The experiment was carried out during 2012 started at February. Whole stalk canes from 12 months old sugarcane genotypes under study were cut into internodes each one have single bud, and five buds were planted per pot. The pots were irrigated regularly every three days until treatment was applied after 8 weeks of planting. After then, the irrigation regime was applied during the next four months as two treatments, control; irrigated every 3 days; and water stress (drought) treatment; irrigated every 15 days. The experiment was designated in a complete randomized block design with three replications. At the end of the experiment, stalk length, diameter, number and volume in addition to Brix% were determined. total chlorophyll, chlorophyll-a and chlorophyll b were determined as described by Arnon (1949). Stalk volume (cm^3) was determined by using the following formula; Stalk volume (cm^3) = $\frac{1}{4}\pi LND^2$ where L = stalk length, N = stalk Number and D = stalk diameter. The drought tolerance index (D.T.I.) was determined by calculating the mean performance of stalk length, diameter, number and volume in addition to Brix% as described by Reddy and Vaidyanath (1986).

Isolation of Sugarcane DNA:

The total DNA isolation from fresh sugarcane leaf roll was carried out according to Dellaporta modified protocol (Dellaporta *et al*., 1983). Five stalks of sugarcane were collected from different individuals of a single genotype. 100 mg of the soft inner leaf roll tissues were weighed out, frozen and were manually ground into a powdery consistency in liquid nitrogen to a fine powder using mortar and pestle.The powdery samples were then placed inside a 50-ml centrifuge tube and stored at -80^0C freezer until DNA extraction. DNA was extracted according to the protocol described by Dellaporta *et al*., (1983). Concentrations of extracted DNA were estimated by known concentration of Lambada DNA in 1% (W/V) agarose gel.

PCR primers design:

TRAP is a simple, 2-primer polymerase chain reaction (PCR) technique. The design of fixed primers was based on the method reported by Hu and Vick (2003). The forward (fixed) primer was designed from genes or EST sequences and the accompanied reverse (arbitrary) primer was designed to target introns (AT rich) or exons (GC rich) (Li and Quiros, 2001; Vettore *et al*., 2001). Both primers are usually about 18 bp long. Two fixed primers designed from candidate genes involved in the drought tolerance response metabolism (Aquaporin, Aqua; and dehydration binding factor, DBF) were used in the present investigation. The primers were designed on the basis of the web-based PCR primer design software Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) using the following parameters: a primer optimal T*m*, maximum T*m*, and minimum T*m* of 53, 55 and 50^oC, re-

spectively, and a GC content between 40 and 60%. The sequence information of three arbitrary primers used in this study was provided by Li and Quiros (2001). The primer details are described in Table 2.

PCR Protocol:

TRAP reactions were performed based on the protocol of Hu and Vick (2003). Fixed primers were combined with each of three arbitrary primers for a total of 6 primer combinations. Each reaction was carried out in a total volume of 20/µL containing 2/µL of 10x PCR buffer, 0.5/µL of 100 mM $MgCl_2$, 1.0/µL each of 10/pmol fixed and arbitrary primers, 1./µL of 10mM dNTPs (Promega, Madison, WI USA), 0.35/µL of 5U *Taq*polymerase (Promega, Madison, WI USA) and 1.0/µL of 50ng genomic DNA and in the final added 13.15/µL of nuclease free water to complete the total reaction . The conditions for PCR were as follows: an initial denaturing step was performed at 94°C for 4 min followed by 5 cycles at 94°C for 45 s, 35°C for 45 s and 72°C for 1 min, followed by 35 cycles at 94°C for 45 s, 53°C for 45 s and 72°C for 1 min with a final extension step at 72°C for 7 min. All the PCR reactions were performed on a My Gene™ series peltire thermal cycler Model MG 96G (long Gene®).

Electrophoresis:

After PCR, the amplified products were run on 4 % agarose gel in 0.5X TBE buffer. Fourgrams of agarose was dissolved in 100 ml of TBE buffer (100 mM Tris, 90 mM boric acid, and 1 mM EDTA) and boiled in a microwave oven. Agarose medium was then cooled down to about 60°C. Ethidium bromide was added before pouring the gel in the submarine electrophoresis unit, to give a final concentration of 0.5 µg/ml of Eth Br. Gel was left to solidify at room temperature, TBE buffer was added to fill the electrode chamber, loading buffer (1 ml glycerol, 10 mM Na_2EDTA, 50 mg SDS, 100 mg bromophenol blue, 13 mg xylene cyanol and water up to50 ml) was added to 15µl of PCR product and loaded in the gel. One Kbp DNA ladder was used as a standard. The electrophoresis run was performed at 80 V in DNA electrophoresis unit (Biometra) for120 to 150 mins. Obtained DNA bands were visualized using UV-trans-illuminator and photographed by gel documentation (Bio-docanalyze). Digital tiff images were derived from the software. The images obtained from each agarose gel was scored manually as present (1) and absent (0).

Data analysis:

TRAP-based molecular markers were scored visually using the software package MVSP (Multi-Variate Statistical Package) and DNA bands were scored as present (1) or absent (0). The pairwise comparisons between the tested isolates were used to calculate the coefficient of genetic similarity (GS) and distance (GD) matrix according to Nei and Li (1979). Cluster analysis was presented as the dendrogram based on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA).

Results and Discussion:

I- Agronomic Traits:

Data regarding the average performance of all genotypes for stalk length, diameter, number, volume and Brix% are presented in Table (3), while those for total chlorophyll, Chlorophyll-a and Chlorophyll-b are found in Table (4). Generally, all studied characters, except chlorophyll in some cases, were sharply affected by drought stress in comparison to the control treatment, however,

highly significant differences between the tested genotypes were observed. The analysis of variance (Table 5) revealed significant differences in all studied traits for genotypes, and drought stress.

Stalk height:

Stalk height ranged from 70.64 cm in EH 16-9 to 102.2 cm in G99-103 under control treatment (Table 3). While under drought stress, stalk length ranged from 52.93 cm in PH8013 to 72.67 cm in EI 8-129. The genotype EI 8-129 followed by EI 264-2, EI 24-3 and EH 16-9 was less affected under drought conditions. While, G99-103, N26, NCO310, G2000 -79 and PH8013 were the most drought sensitive genotypes which displayed the highest % reduction, as compared to the control treatment. Cane elongation and stalk height are negatively and strongly affected by drought conditions (Da Silva & Da Costa, 2004; Soares *et al.*, 2004 and Inman-Bamber & Smith, 2005). According with Domaingue (1995), Soares *et al.* (2004) and Silva *et al.* (2008), stalk height is the most severely reduced parameter under drought conditions.

Stalk diameter:

stalk diameter ranged from 1.45 cm in EI 264-2 to 2.64 cm in EH 16-9 under control treatment (Table 3). While, it was affected by drought stress and ranged from 0.79 cm in G2000 -79 to 1.76 cm in EH 16-9. The less affected genotypes were EI 264-2, EI 24-3 and EI 8-129 while G2000 -79, NCO310 and G.T.54C-9 were the most drought sensitive genotypes, as compared to the control treatment. Similar results were also observed by Khan *et al.*, (2013), who found decrease in the cane girth under water stress condition. In contrast, Silva *et al.*, (2008) reported that in water stress condition cane girth increases as compared to well watered crop. The results in the present study were contrary to their results and displayed decrease in the stalk diameter under water stress condition.

Stalk number:

Stalk number ranged from 7.67 in G99-103 to 11.67 in N26 under control treatment, while it ranged from 5.0 stalks in NCO310 and PH8013 to 8.33 stalks in EI 8-129 under drought stress (Table 3). EI 8-129 was less affected under drought conditions which displayed the least reduction (7.44 %) in stalk number as compared to the control treatment. While, the highest reduction in stalk number was obtained by NCO310 (50% of the control). The other genotypes displayed variable percentages of reduction in stalk number under drought stress ranged from 48.59% in N26 to 30.51% in G99-103 (Table 3). Similar results were also obtained by Bendigeri *et al.* (1986), Johari *et al.* (1998), who observed significant reduction in millable canes under drought stress. Varsha and Lakhidive (1997) observed that maximum number of millable canes was in normal irrigation and 26.7% reduction in millable canes due to water stress. Ramesh and Mahadevaswamy (2000) studied on the effect of drought during formative phase and reported reductions in the total number of shoots and their conversion to millable canes at harvest, cane length and number of internodes.

Stalk volume:

Under control treatment, the highest volume of stalks was recorded in G.T.54C-9 (4181.39 cm^3) followed by EH 16-9 (3866.34 cm^3) and N26 (3660.42 cm^3 cm^3) (Table3). While the least stalk volume was found in EI 264-2 (1469.56). This

vegetative growth of the genotypes was reduced under drought stress in which the lowest volume of stalks was observed in G84 – 47 (587.33 cm^3) and EI 264-2 (591.58 cm^3) while N26 (1707.30 cm^3), G.T.54C-9 (1483.24 cm^3) and PH8013 (1301.59 cm^3) displayed the highest stalk volume. Similar results were also obtained by Singh & Reddy , 1980; Wiedenfeld, 1995; Ramesh, 2000; Ramesh & Mahadevaswamy, 2000; Da Silva & Da Costa, 2004; Soares *et al*., 2004 and Inman-Bamber & Smith, 2005.

Brix %:

Under control treatment, the highest percentage of Brix was recorded in EH 16-9 (19.49%) followed by PH8013 (18.34%) G84 – 47 (18.24%) and EI 264-2 (18.05%) (Table 3). While, the least Brix% was found in NCO310 (16.03%) and EI 8-129 (16.17%). The percentage of Brix was reduced under drought stress in which the highest Brix% was found in G.T.54C-9 (16.57 %) followed by PH8013 (16.29 %) and G84 – 47 (16.13 %). The lowest Brix% was recorded in N26 (14.51 %) followed by EI 264-2 (14.65 %) and G99-103(14.89 %). Singh and Reddy (1980) observed a greater reduction in sucrose percent in juice and with increased reducing sugars under soil moisture stress conditions. Under optimum soil moisture conditions, the quality of juice was found to be ideal as compared to stress conditions (Parameshwaran *et al.*1987).

Drought tolerance index (D.T.I.):

Generally, plant tolerance is a measure of the plant's ability to survive and even to grow proactively in the presence of stress (Srivastava *et al*., 2012). Normally, drought tolerant sugarcane genotypes are selected depending upon certain well defined morphological and physio-biochemical characteristics related to tolerance under natural field conditions (Zhou *et al*., 2011). In the present study, drought tolerance index (D.T.I), which is based on the mean performance of stalk height, diameter, number, volume and brix%, indicated that EI 8-129 (DTI=82.20) followed by EI 24-3 (DTI=74.25) were the most tolerant genotypes (Table 3). This would indicate that these genotypes are relatively drought tolerance and could be used in breeding programs to improve sugarcane.

Meanwhile, NCO310 (DTI=56.39) followed by G2000 -79 (DTI=56.47) were the most sensitive genotypes. The other genotypes displayed DTI ranged from 62.43 in G84 – 47 to 68.59 in EI 264-2. Patil (2008) reported that various morphological characters, such as plant height, leaf area, number of tillers, internodal length, girth of internodes and dry matter production showed significant decrease due to water stress treatments.

Chlorophyll content:

Under control treatment (Table 4), the lowest content of chlorophyll was observed in G84 – 47 (3.21 mg/g Fr.Wt.) for Chl.a, and in EI 8-129 for Chl.b. (2.41 mg/g Fr.Wt.) and total Chl. (5.02 mg/g Fr.Wt.). While, the highest content was recorded in N26 for Chl.a (17.22 mg/g Fr.Wt.), Chl.b. (34.75 mg/g Fr.Wt.) and total Chl. (36.27 mg/g Fr.Wt.). Under drought stress, EI 8-129 revealed the minimum reduction in chl.a (4.31%) and total Chl. (22.91%) while, EH 16-9 displayed least reduction for Chl.b (0.65%). The maximum reduction in chlorophyll pigments under drought stress was found in NCO310 (79.46%) for Chl.a, G.T.54C9 for

Chl.b (89.87%) and total Chl. (92.09%). Similar results were reported in sugarcane (Joshi and Naik 1980; Chandra 1993, Silva *et al.*, 2007 and Jangpromma *et al.*, 2010, Ecco *et al.*, 2013).The tolerant genotype maintained stability of chloroplast and injury was also marginal. Du *et al.*(1996) noticed that during water stress, chlorophyll content and total soluble proteins decreased linearly with decreasing leaf water potential.

On contrary, the content of chl.a, Chl.b and total Chl. increased in EI 264-2, G84 – 47 and EI 266-2 under drought stress as compared to the control treatment (Table 4). In most plant species, chlorophyll is generally sensitive to drought stress, however, drought can increase chlorophyll content in some cases (Mensah *et al.*, 2006).

II- TRAP markers based on Aqua and DBF candidate drought genes:

Polymorphisms and genetic variability in drought tolerance genes within 12 genotypes of *Saccharum officinarum* were evaluated using TRAP analysis. For the drought tolerance candidate genes, 6 TRAP primer combinations using two fixed forward primers of Aquaporin (Aqua) and Dehydration binding factor (DBF) loci and three arbitrary reverse primers, gave a total of 80 DNA bands, with a mean of 13.3 bands per primer combination (Table 6 and Fig. 1). The Aqua gene showed the high number of amplified (55 bands) bands than DBF locus (25 bands). In addition, the Aqua gene showed the highest percentage of polymorphic (37 bands, 67.27%) bands than DBF locus (20 bands, 80%) with 71.25% overall polymorphism. The largest number of amplified fragments was observed with the Aqua + arbit.2 primer combination (20 bands), and the lowest (7 bands) with the DBF + arbit.1. Occurrence of a highly polymorphic band profile in sugarcane can be attributed to the fact that sugarcane is a highly self-incompatible, cross-pollinating, complex polyploid grass species with homologous and homeologous chromosomes (D'hont *et al.*, 1995; Ming *et al.*, 2001).

Significant correlations were observed between D.T.I. and number of amplified fragments of Aqua ($r=0.758$, $P<0.01$) and DBF ($r=0.660$, $P<0.01$) loci. These results indicated that these loci play an important role in sugarcane drought tolerance. In this instance, the most tolerant genotypes EI 8-129 (DTI=82.20) and EI 24-3 (DTI=74.25) revealed the highest number of amplified DNA fragments, 57 and 55 bands, from both loci, respectively (Tables 3 and 6). While, G2000 -79 (DTI=56.47) was the most drought sensitive genotype and amplified the least number of Aqua+DBF fragments (35 bands). These results also reflected the feasibility and effectiveness of these loci as molecular markers for drought tolerance in sugarcane. Creste *et al.* (2010) amplified 60 Aqua and 47 DBF bands from 60 sugarcane genotypes by three primer combinations for each locus. They found that the largest number of polymorphic fragments was obtained with the Aqua/Arb2 (24 fragments) and DBF/Arbit.1 (17 fragments) primer combinations. Promising observations from the evaluation of aquaporins among stress resistance or sensitive plants, such as drought susceptible and drought tolerant wheat cultivars, (Morillon and Lassalles, 2002), sugarcane genotypes (Creste *et al.*, 2010), other crop cultivars (Lian

et al., 2004), or stressed EST libraries (Houde *et al*., 2006), clearly indicate that aquaporins would be important for water uptake, transport and identification or development of any stress tolerant genotypes of crop species.

The transcription factors act on dehydration-responsive *cis*-acting element to trigger gene expression in an ABA- independent pathway (Shinozaki & Yamaguchi-Shinozaki 2007). Transcription factors expressed by sugarcane plants indicate that ABA-dependent and ABA- independent pathways are presented in sugarcane responses to water deficit (Iskandar *et al*., 2011). Once activated, transcription factors act as DNA-binding proteins, which are capable of mediating the transcription of key proteins in the stress response mechanism. The relative abundance of transcripts of the 51 genes in different parts of mature sugarcane plants of cultivar Q117 was determined by Iskandar *et al*. (2011) using Real Time quantitative PCR (RTqPCR) analysis of total RNA samples. They found that dehydrin transcripts were dramatically induced by water stress, up to 1000-fold.

It is accepted that crosses between unrelated genotypes will maximize the number of segregating alleles, resulting in a large genetic variance in the progeny (Messmer *et al*. 1993), thereby increasing the opportunity for selecting rare genotypes that may be superior (Becelaere *et al*. 2005). TRAP markers are derived from candidate genes representing functional markers that may be directly involved with a phenotypic trait variation. In the present study, specific regions of the sugarcane genome related to drought tolerance (Aqua and DBF loci), rather than the entire genome, were sampled to evaluate the genetic variability of the important sugarcane genotypes. The results revealed that the lowest similarity value for drought was obtained between the drought tolerant genotype EI 24-3 and the most sensitive one G2000-79 (0.622, Table 7). The most tolerant genotype EI 8-129 also showed low similarity (0.639) with the sensitive EH 16-9 one. These results illustrating that these crosses would probably result in the highest variability for drought among the genotypes sampled. It has been suggested that genetic diversity estimation for planning crossing purposes should be done based on candidate genes for specific traits (Liu *et al*. 2004; Alwala *et al*. 2006). The results also revealed that the highest genetic similarity was 0.957 between the sugarcane genotypes NCO310 and N26.

The dendrogram tree (Fig. 2) showed that the 12 sugarcane genotypes were clustered together in main groups of clusters within a branched-off 0.721 genetic similarity (GS). The 1st group contained two sub-clusters within a branched-off 0.751 GS. In the first sub-cluster, El 266-2 and N26 were clustered together firstly at 0.957 GS and then with G99-103 at GS 0.893 followed by the most drought sensitive genotype G2000-79 within a branched-off 0.784 GS. The 2nd sub-cluster contained the most drought tolerant genotypes El 8-129, El 264-2 and El 24-3 within 0.818 GS. The 2nd group contained two sub-clusters within a branched-off 0.740 GS. The first sub-cluster contained three genotypes that have NCO310 common parent, in which G84-47 and NCO310 were clustered together firstly at 0.902 GS and then with 54C9 at 0.805 GS. The 2nd sub-cluster

contained PH8013 and EH 16-9 within 0.840 GS.

Cluster analysis based on TRAP markers of Aqua and DBF loci related to drought tolerance displayed that some genotypes that have common parents were grouped together (varieties G84-47, NCO310 and 54C9. In addition, the drought tolerant genotypes were also grouped in the same cluster. Similar results were also obtained by Alwala *et al*. 2006 and Creste *et al*., 2010. It has been suggested that the measure of genetic diversity by molecular markers for breeding purposes should be based on functionally characterized genes, or targeted genes, as these may reflect functional polymorphisms (Andersen and Lu¨bberstedt 2003; Ramalingam *et al*.2003).

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Table (1): The studied sugarcane genotypes and their parents.

Genotype	Parents	
	♂	♀
G.T.54C-9[a]	F37-925 (Pas 38X F83)	NCo.310
N:CO.310	CO321	CO421
EI 24-3	BU459	PR1117
EH 16-9	LCP81-30	CP81-325
EI 8-129	BT1562	B1-2
EI 264-2	MP1723-94	BU794
EI 266-2	MP1731-94	LCO382
PH8013	Phil.64-2227	CAC71-312
G84 – 47	open	NCo.310
G99-103	Us.74-3	CP.76-1053
G2000 -79	Ministry of Agriculture	
N26	Ministry of Agriculture	

Table (2): Sequence data of two fixed primers and three arbitrary primers used to estimate the genetic variability in sugarcane genotypes.

Primer	Sequence (5` → 3`)	GenBank ID	Sequence ID	References
Fixed primer				
Aqua	ATCTCCGGCGGCCACAT	CA086489	Water channel	Vettore *et al*. (2001)
DBF	CTCTGCCACCACCACCTC	CA077947	Transcription factor	Vettore *et al*. (2001)
Arbitrary primer				
Arbit.1	GACTGCGTACGAATTAAT			Li and Quiros (2001)
Arbit.2	GACTGCGTACGAATTGAC			Li and Quiros (2001)
Arbit.3	GACTGCGTACGAATTTGA			Li and Quiros (2001)

Table (3): Mean values of stalk length (cm), diameter (cm), number, volume (cm3) and Brix% in addition to drought tolerance index (D.T.I.) among 12 sugarcane genotypes grown under control and drought stress conditions.

Genotypes	Stalk height		Stalk diameter		Stalk number		Stalk volume		Brix %		D.T.I.
	Control	Drought stress	Control	Drought stress	Control	Drought stress	Control	Drought stress	Control	Drought stress	
EH 16-9	70.64	62.47 11.57%*	70.64	62.47 11.57%	10.00	5.67 43.30%	3866.34	906.14 76.56%	19.49	15.17 22.17%	62.61
EI 8-129	76.97	72.67 5.59%	76.97	72.67 5.59%	9.00	8.33 7.44%	1862.85	1000.96 46.27%	16.17	15.95 1.36%	82.20
EI 264-2	78.07	70.50 9.70%	78.07	70.50 9.70%	10.00	5.33 46.70%	1469.56	591.58 59.74%	18.05	14.65 18.84%	68.59
EI 266-2	86.53	71.07 17.87%	86.53	71.07 17.87%	8.67	5.67 34.60%	1879.48	624.28 66.78%	17.35	15.47 10.84%	64.60
EI 24-3	74.27	66.20 10.87%	74.27	66.20 10.87%	10.33	6.33 38.72%	1745.65	1013.57 41.94%	17.44	15.60 10.55%	74.25
G84 - 47	79.73	64.08 19.63%	79.73	64.08 19.63%	10.67	6.00 43.77%	1868.45	587.33 68.57%	18.24	16.13 11.57%	62.43
G99-103	102.2	69.88 31.63%	102.2	69.88 31.63%	7.67	5.33 30.51%	2077.92	1080.50 48.00%	17.30	14.89 13.93%	67.10
G2000 -79	95.47	66.59 30.26%	95.47	66.59 30.26%	9.33	5.33 42.87%	2892.11	843.96 70.82%	17.45	15.31 12.26%	56.47
G.T.54C-9	88.00	66.40 24.55%	88.00	66.40 24.55%	10.67	7.00 34.40%	4181.39	1483.24 64.53%	17.41	16.57 4.82%	63.37
NCO310	78.93	54.86 30.50%	78.93	54.86 30.50%	10.00	5.00 50.00%	3035.79	664.76 78.10%	16.03	15.35 4.24%	56.39
N26	77.77	53.55 31.15%	77.77	53.55 31.15%	11.67	6.00 48.59%	3660.42	1707.30 53.36%	17.34	14.51 16.32%	64.07
PH8013	75.27	52.93 52.93%	75.27	52.93 29.68%	8.00	5.00 37.50%	3025.26	1301.59 56.98%	18.34	16.29 11.18%	63.76
MEAN	81.99	64.27 21.08%	81.99	64.27 21.08%	9.67	5.92 38.20%	2630.43	983.77 60.97%	17.55	15.49 11.51%	

* % Reduction of the control

Table (4): Mean values of Chlorophyll-a, Chlorophyll-b and total Chlorophyll (mg/g Fr.Wt.) among 12 sugarcane genotypes grown under control and drought stress conditions.

Genotypes	Chlorophyll-a			Chlorophyll-b			Total Chlorophyll		
	Control	Drought stress	% Reduction of the control	Control	Drought stress	% Reduction of the control	Control	Drought stress	% Reduction of the control
EH 16-9	9.44	8.97	4.98	12.35	12.27	0.65	17.90	11.69	34.69
EI 8-129	3.94	3.77	4.31	2.41	3.97	--	5.02	3.87	22.91
EI 264-2	4.73	9.51	--	3.57	12.48	--	7.62	11.93	--
EI 266-2	4.77	7.99	--	4.39	9.10	--	7.07	8.80	--
EI 24-3	8.58	3.94	54.08	6.73	5.18	23.03	11.67	4.95	57.58
G84 - 47	3.21	5.68	--	7.75	9.21	--	7.33	8.67	--
G99-103	12.41	8.78	29.25	22.06	9.21	58.25	24.79	8.97	63.82
G2000 -79	14.95	10.10	32.44	26.25	12.32	53.07	30.94	11.85	61.70
G.T.54C-9	14.85	3.80	74.41	26.94	2.73	89.87	35.04	2.77	92.09
NCO310	13.58	2.79	79.46	25.23	4.13	83.63	30.14	3.92	86.99
N26	17.22	4.88	71.66	34.75	3.53	89.84	36.27	3.58	90.13
PH8013	16.95	3.60	78.76	26.75	4.37	83.66	35.06	4.20	88.02
MEAN	10.39	6.15	40.81	16.60	7.38	55.54	20.73	7.1	34.69

Table (5): Analysis of variance for stalk length, diameter, number and volume, in addition to Brix%, Chlorophyll-a, Chlorophyll-b and total Chlorophyll in 12 sugarcane genotypes grown under control and drought stress conditions.

S.V.	DF	Stalk height	Stalk diameter	Stalk number	Stalk volume	Brix %	Chlo.a	Chlo.b	Total Chlo.
		MS	MS	MS	MS	MS	MS	MS	MS
Replicates	2	77.075	0.161	4.875	923015.49	6.676**	20.262	25.307	233.018*
Genotypes	11	278.884**	0.421*	5.425*	2134743.21*	1.880**	43.809**	171.916**	206.061**
Drought stress	1	5652.680**	12.525**	253.125**	48856710.34**	76.385**	323.003**	1531.350**	3346.575**
G x D	11	127.314**	0.164	2.731	866278.18	1.971**	61.539**	266.385**	305.566**
Error	46	45.964	0.177	2.295	1043612.86	0.339	13.815	32.674	51.075

Table (6): Number of amplified DNA-fragments, polymorphic bands and % of polymorphism, in twelve sugarcane genotypes investigated with TRAP markers of Aqua and DBF loci.

Fixed primer	Arbitrary primer	No. of amplified bands												Total amplified bands	No. of polymorphic bands	% of polymorphism
		G2000 -79	N26	EI 266-2	G99-103	G84 – 47	NCO310	54C9	EI 8-129	EI 264-2	EI 24-3	PH8013	EH 16-9			
Aqua	TRAP arbit-1	9	8	10	11	8	6	8	12	12	10	6	8	17	12	70.59
	TRAP arbit-2	12	14	13	14	14	14	10	19	13	13	11	9	20	13	65.00
	TRAP arbit-3	7	7	8	9	10	10	11	14	14	15	12	10	18	12	66.67
Total		28	29	31	34	32	30	29	45	39	38	29	27	55	37	67.27
DBF	TRAP arbit-1	2	6	6	5	2	2	1	1	1	5	2	2	7	6	86.00
	TRAP arbit-2	2	5	5	5	5	5	8	7	9	10	6	4	10	8	80.00
	TRAP arbit-3	3	5	5	4	3	3	3	4	4	2	3	7	8	6	75.00
Total		7	16	16	14	10	10	12	12	14	17	11	13	25	20	80.00
Grand TOTAL		35	45	47	48	42	40	41	57	53	55	40	40	80	57	71.25

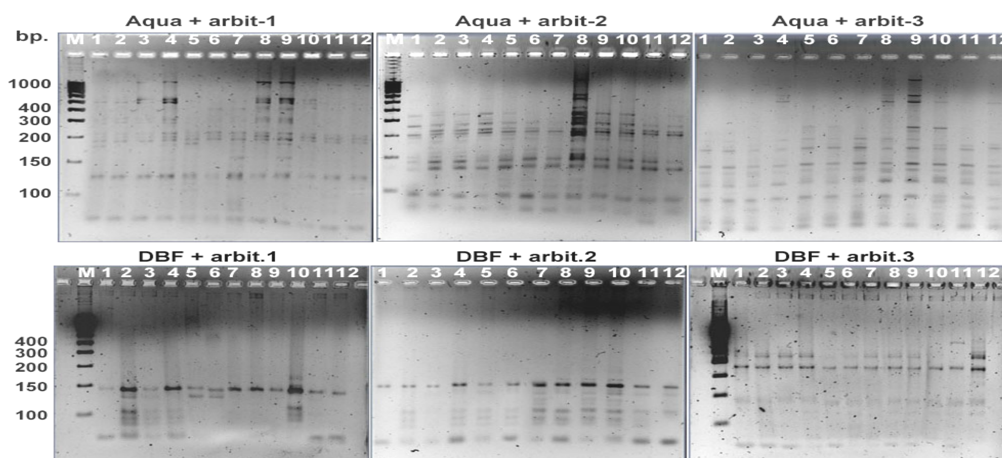

Fig. 1. TRAP profile of sugarcane genotypes using Aqua and DBF loci; M=DNA marker, (1) G2000-79, (2) N26, (3) EI 266-2, (4) G99-103, (5) G84-47, (6) NCO310, (7) G.T.54C9, (8) EI 8-129, (9) EI 264-2, (10) EI 24-3, (11) PH8013, and (12) EH 16-9.

Table (7): Genetic similarity (below the diagonal) and distance (above the diagonal) values calculated from the TRAP markers amplified from twelve sugarcane genotypes by three primer combinations of Aqua and DBF loci.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12
1- G2000 -79	1.000	0.223	0.248	0.260	0.283	0.328	0.459	0.332	0.319	0.475	0.341	
2- N26	0.800	1.000	0.044	0.126	0.246	0.223	0.395	0.268	0.281	0.329	0.296	
3- EI 266-2	0.780	0.957	1.000	0.100	0.269	0.246	0.417	0.238	0.248	0.268	0.257	
4- G99-103	0.771	0.882	0.905	1.000	0.311	0.288	0.428	0.223	0.208	0.278	0.330	
5- G84 – 47	0.753	0.782	0.764	0.733	1.000	0.103	0.260	0.292	0.277	0.386	0.260	
6- NCO310	0.720	0.800	0.782	0.750	0.902	1.000	0.174	0.243	0.284	0.334	0.268	
7- 54C9	0.632	0.674	0.659	0.652	0.771	0.840	1.000	0.365	0.294	0.316	0.248	
8- EI 8-129	0.717	0.765	0.788	0.800	0.747	0.784	0.694	1.000	0.136	0.190	0.271	
9- EI 264-2	0.727	0.755	0.780	0.812	0.758	0.753	0.745	0.873	1.000	0.183	0.294	
10- EI 24-3	0.622	0.720	0.765	0.757	0.680	0.716	0.729	0.804	0.833	1.000	0.260	
11- PH8013	0.711	0.744	0.773	0.719	0.771	0.765	0.780	0.714	0.745	0.771	1.000	
12- EH 16-9	0.667	0.682	0.713	0.705	0.707	0.700	0.716	0.639	0.667	0.674	0.840	1.00

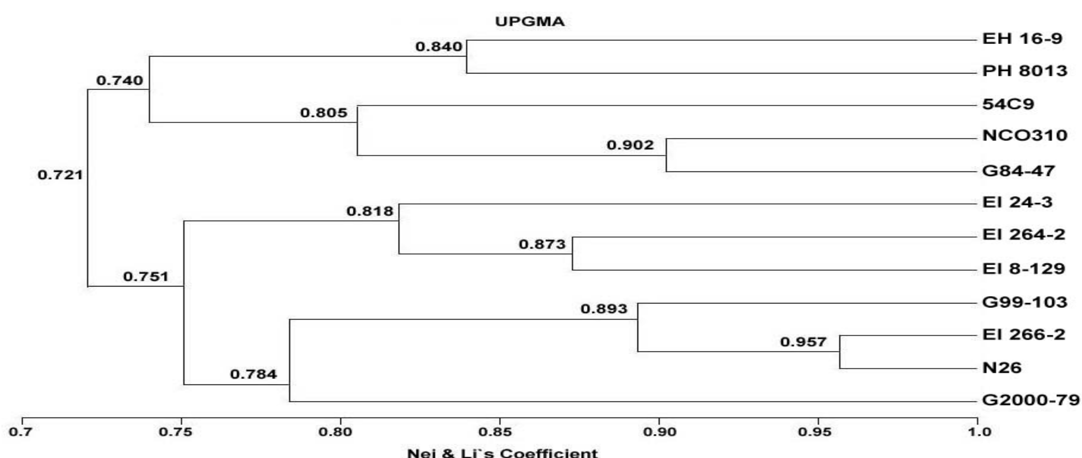

Fig. (2): Dendrogram demonstrating the relationships among 12 sugarcane genotypes based on data recorded from polymorphism of TRAP markers of Aqua and DBF genes.

الاختلافات الوراثية في قصب السكر بناء علي واسمات جينات تحمل الجفاف وبعض الصفات المحصولية

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استهدفت الدراسة تقييم أداء 12 تركيب وراثي من قصب السكر تحت اجهاد الجفاف ، وبالإضافة إلي ذلك تمت دراسة الاختلافات الوراثية بين هذه التراكيب الوراثية علي اساس التعدد المظهري باستخدام واسمات الـ TRAP الخاصة بجينات تحمل الجفاف DBF ، Aqua. أوضحت النتائج ان صفات طول العود ، قطر العود ، وعدد عيدان القصب وحجمها بالإضافة إلي معامل بركس قد تأثرت بشكل حاد بسبب معاملة اجهاد الجفاف مقارنة بمعاملة الكنترول ، بينما كان هناك اختلافات معنوية بين التراكيب الوراثية المختبرة. وقد اظهر معامل تحمل الجفاف ان التركيب الوراثي EI 8-129 وبلية EI 24-3 كانت أكثر تحملا للجفاف دون غيرها ، بينما NCO310 ، G2000-79 كانت أكثرها حساسية للجفاف. وقد خفضت معاملة اجهاد الجفاف محتوي الكلوروفيل في معظم التراكيب الوراثية المختبرة بينما أدت لزيادتها في EI 264-2 ، G84 – 47 ، EI 266-2 .

اظهر جين اكوا Aqua اكبر عدد من شظايا الـ DNA متعددة المظهر مقارنة بالموقع DBF بمتوسط عام 71.25% تعدد مظهري. اكبر عدد من مقاطع الـ DNA تم الحصول عليها من توليفة البادئ Aqua + arbit.2 ، بينما كان اقل عدد من الحزم تم الحصول عليه بواسطة DBF + arbit.1. التلازم المعنوي بين معامل تحمل الجفاف وعدد الحزم الناتجة من جينات DBF ، Aqua تدل علي ان هذه الجينات تلعب دورا هاما في تحمل قصب السكر للجفاف. وقد لوحظ انخفاض التماثل الوراثي بين التراكيب المتحملة للجفاف وتلك الحساسة للجفاف مما يدل علي ان التهجين بين هذه التراكيب ينشأ عنه تباينات وراثية عديدة لتحمل الجفاف. وقد اوضح التحليل العنقودي لواسمات الـ TRAP الخاصة بجينات DBF ، Aqua ان التراكيب الوراثية المشتركة في احد الاباء قد تجمعت سويا في نفس المجموعة العنقودية ، وبالمثل التراكيب الوراثية التي أظهرت تحملا للجفاف قد تجمعت في مجموعة واحدة ومن ثم فقد أوضحت الدراسة فعالية وجدوي استخدام جينات DBF ، Aqua للحصول علي واسمات وراثية لتحمل الجفاف في قصب السكر.